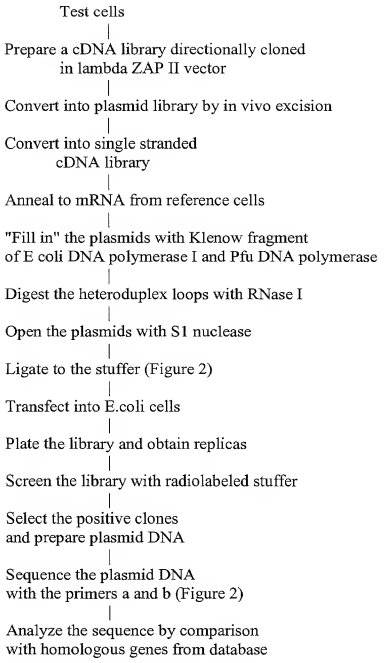
Test cells
Prepare a cDNA library directionally cloned in lambda ZAP II vector
Convert into plasmid library by in vivo excision
Convert into single stranded cDNA library
Anneal to mRNA from reference cells
"Fill in" the plasmids with Klenow fragment of E coli DNA polymerase I and Pfu DNA polymerase
Digest the heteroduplex loops with RNase I
Open the plasmids with S1 nuclease
Ligate to the stuffer (Figure 2)
Transfect into E.coli cells
Plate the library and obtain replicas
Screen the library with radiolabeled stuffer
Select the positive clones and prepare plasmid DNA
Sequence the plasmid DNA with the primers a and b (Figure 2)
Analyze the sequence by comparison with homologous genes from database

Figure 1


a
5'- CTATAGTGTCACCTAAATA-3' (SEQ ID NO. 1)
MluI
NruI
(SEQ ID NO. 2)
GGGTTTTCTATAGTGTCACCTAAATAACGCGTCGACGTCGCGATCCCTTTAGTGAGGGTTAATGGGTTTT
CCCAAAAGATATCACTGTGGATTTATTGCGCTGCTGCAGCGCTAGGGAAATCACTCCCAATTACCCAAAA
(SEQ ID NO. 3)
3'-GATATCACTGTGGATTTAT-5'
3'-AGGGAAATCACTCCCAATTA-5'
SP6 polymerase promoter primer (SEQ ID NO. 12)
b
(SEQ ID NO. 4)

Figure 2

ATGTGTGGGGTTTTCTA-STUFFER-AATGGGTTTTGATTGAAGCT (SEQ ID NO. 5)

	Ile Cys Ile Glu Ala (SEQ ID NO. 6)
Sequence obtained by SDBC:	ATG TGT G-G ATT GAA GCT (SEQ ID NO. 7)
Sequence obtained by RT PCR from MCF7 cells:	Ile Cys Val Ile Glu Ala (SEQ ID NO. 8) ATG TGT GTG ATT GAA GCT (SEQ ID No. 9)
Sequence obtained by RT PCR from normal breast cells:	Ile Cys Glu Ile Glu Ala (SEQ ID NO. 10) ATG TGT GAG ATT GAA GCT (SEQ ID NO. 11)

Figure 3